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Identification of two apurinic/apyrimidinic endonucleases from *Caenorhabditis elegans* by cross-species complementation

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Abstract

The *Saccharomyces cerevisiae* mutant strain YW778, which lacks apurinic/apyrimidinic (AP) endonuclease and 3′-diesterase DNA repair activities, displays high levels of spontaneous mutations and hypersensitivities to several DNA damaging agents. We searched a cDNA library derived from the nematode *Caenorhabditis elegans* for gene products that would rescue the DNA repair defects of this yeast mutant. We isolated two genes, *apn-1* and *exo-3*, encoding proteins that have not been previously characterized. Both APN-1 and EXO-3 share significant identity with the functionally established *Escherichia coli* AP endonucleases, endonuclease IV and exonuclease III, respectively. Strain YW778 expressing either *apn-1* or *exo-3* shows parental levels of spontaneous mutations, as well as resistance to DNA damaging agents that produce AP sites and DNA single strand breaks with blocked 3′-ends. Using an in vitro assay, we show that the *apn-1* and *exo-3* genes independently express AP endonuclease activity in the yeast mutant. We further characterize the EXO-3 protein and three of its mutated variants E68A, D190A, and H279A. The E68A variant retains both AP endonuclease and 3′-diesterase repair activities in vitro, yet severely lacks the ability to protect strain YW778 from spontaneous and drug-induced DNA lesions, suggesting that this variant E68A may possess a defect that interferes with the repair process in vivo. In contrast, D190A and H279A are completely devoid of DNA repair activities and fail to rescue the genetic instability of strain YW778. Our data strongly suggest that EXO-3 and APN-1 are enzymes possessing intrinsic AP endonuclease and 3′-diesterase activities.

Keywords: DNA repair; Base-excision; *C. elegans*; Yeast; Enzymes; Mutations

1. Introduction

DNA base moieties can be modified by endogenous chemical reactions as well as by environmental factors. These modified bases can be excised by DNA glycosylases in the first step of the base-excision repair (BER) pathway, to generate a secondary DNA lesion, i.e., an apurinic/apyrimidinic (AP) site [1,2]. AP sites also arise as a result of spontaneous base loss [3]. The mutagenic potential of AP sites is prevented by AP endonucleases, which catalyze the second enzymatic step of the BER pathway. In *Escherichia coli*, two hydrolytic AP endonucleases, endonuclease IV and exonuclease III, have been well characterized [4]. Both enzymes recognize AP sites and cleave the phosphodiester bond 5′ to the AP site, generating a 3′-hydroxyl group and a 5′-deoxyribose phosphate terminus [4,5]. These enzymes also remove 3′-blocking groups (e.g., 3′-unsaturated aldehydes and 3′-phosphates) to generate a 3′-hydroxyl group [4,5]. Endonuclease IV and exonuclease III exemplify two distinct families of AP endonucleases, termed Endo IV and Exo III [4,6] and both family members are also conserved in yeast [7,8] while only members of the Exo III family have been found in mammalian cells, e.g., human Ape1 [9]. In *E. coli*, mutants devoid of AP endonuclease activity are hypersensitive to agents that create AP sites such as methyl methane sulfonate (MMS) [10]. These AP endonuclease-deficient mutants also exhibit very high rates of spontaneous mutations as a consequence of unrepaired AP sites [11].

In *Saccharomyces cerevisiae*, Apn1 is a member of the Endo IV family and is responsible for at least 97% of the

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AP endonuclease and 3′-diesterase activities in this organism [7]. The remaining bifunctional activity is fulfilled by Apn2, a member of the Exo III family, which acts as a backup protein for the repair of AP sites and 3′-blocked ends in yeast mutants lacking Apn1 [8]. Like *E. coli*, yeast mutants lacking Apn1 and Apn2 are viable, but display hypersensitivities when challenged with DNA damaging agents such as MMS, bleomycin, and H_2O_2 [8,12,13]. These yeast mutants also exhibit a very high rate of spontaneous mutations primarily due to unrepaired AP sites [14]. In addition, mutants lacking both Apn1 and Apn2 are either inviable or grow extremely poorly if they also lack proteins that process single strand breaks such as Rad1/Rad10 endonuclease complex of the nucleotide excision repair pathway or the homologous recombination protein Rad52, respectively [15]. It is evident that Apn1 and Apn2 are required to process endogenously generated single strand breaks with blocked 3′-ends [15].

As in *E. coli* and *S. cerevisiae*, the genome of the nematode *Caenorhabditis elegans* contains two genes *apn-1* and *exo-3* predicted to encode proteins APN-1 and EXO-3 that belong to the Endo IV and Exo III families, respectively [16,17] (accession number 3941298). So far, our previous attempts using plasmids to express *apn-1* and *exo-3* in *E. coli* failed to produce functional AP endonuclease activities (Shatilla and Ramotar, unpublished results). Thus, it remains to be determined whether *apn-1* and *exo-3* directly encode proteins with AP endonuclease and 3′-diesterase activities. We have however, recently demonstrated that total extracts derived from purified embryos of *C. elegans* constitutively express a Mg^{2+}-dependent AP endonuclease activity, similar to that of members of the Exo III family [17]. It is possible that this embryonic AP endonuclease is encoded by the *exo-3* gene, but this remains to be verified.

Cross-species complementation studies have shown that overexpression of either *E. coli* endonuclease IV or exonuclease III in yeast cells devoid of AP endonuclease activity can restore resistance to MMS and H_2O_2 [18,19]. Similarly, *S. cerevisiae* Apn1 has been shown to provide partial complementation toward H_2O_2 and MMS, and full resistance toward bleomycin in *E. coli* exonuclease III- and endonuclease IV-deficient double mutants [20]. The human Ape1 protein, a counterpart of exonuclease III, also restores MMS, but not H_2O_2, resistance to AP endonuclease-deficient yeast cells [21]. Considering these observations, we exploited the power of yeast genetics to search a *C. elegans* cDNA library for genes that would encode AP endonuclease activities. Herein, we isolated *apn-1* and *exo-3* as two independent genes that restore to a DNA repair deficient *S. cerevisiae* mutant strain, lacking AP endonuclease/3′-diesterase activities [12], resistance to DNA damaging agents that create AP sites and DNA strand breaks with blocked 3′-ends. Both genes from *C. elegans* also reduced the high spontaneous mutation rate exhibited by the yeast mutant. Moreover, total cell extracts derived from this mutant bearing either the *apn-1* or *exo-3* gene expressed AP endonuclease activity. We further characterized the *exo-3* gene by creating three point mutations by site-directed mutagenesis to produce the variants E68A, D190A, and H279A, corresponding to amino acid substitutions that are known to disrupt the function of the human Ape1 protein [22–27]. We show that each of the mutations E68A, D190A, and H279A in the *exo-3* gene interferes with the ability of the protein to confer to this yeast mutant resistance to MMS, bleomycin, and H_2O_2. Interestingly, while the D190A and H279A variants are devoid of AP endonuclease and 3′-diesterase activities, these activities are present in the E68A mutant. We discuss that EXO-3 and APN-1 function in a *C. elegans* base-excision repair pathway as DNA repair enzymes with AP endonuclease and 3′-diesterase activities.

2. Materials and methods

2.1. Yeast and bacterial strains

The *S. cerevisiae* laboratory strains YW465 (*MATalpha ade2Δ0 his3Δ-200 leu2Δ-1 met15Δ0 trp1Δ-63 ura3Δ0*), which is wild-type for AP endonuclease, 3′-diesterase and 3′-phosphatase activities, its isogenic triple mutant derivative YW778 (*apn1Δ::HIS3 apn2Δ::KanMX4 tpp1Δ::MET15*) and YW607 (*MATalpha his3D200 leu2-3,112pep4::HIS3 ura3-52*) were generously provided by Dr. Tom Wilson (Ann Arbor, Michigan). Strain YW607 was maintained on YPD agar and strains YW465 and YW778 were maintained on YPD agar supplemented with adenine (20 μg/ml). *E. coli* laboratory strain DH5α (used for amplification of plasmids) was maintained on Luria Broth (LB) agar.

2.2. Chemicals

Growth culture reagents were from Difco (Detroit, MI). MMS and hydrogen peroxide were from Sigma (St. Louis, MO). Bleomycin A5 trihydrochloride was from Calbiochem (La Jolla, CA). Pfu polymerase was from Stratagene (La Jolla, CA). Primers were from Invitrogen (Carlsbad, CA) or Biocorp Inc. (Montreal, Que.). All other chemicals were from Sigma unless otherwise stated.

2.3. C. elegans cDNA library

A library from *C. elegans* genomic DNA (Invitrogen), kindly provided by Dr. Matthias Peter (Zurich, Switzerland), consisted of fragments of the *C. elegans* genome directionally cloned downstream of the GAL4 Activation Domain in the ProQuest expression vector pPC86 (bearing TRP1 and AMP^r markers), generating GAL4 AD-cDNA fusion proteins, under the control of the yeast ADH promoter.

2.4. Transformation of yeast and isolation of methyl methane sulfonate-resistant transformants

Yeast strain YW778 was transformed with 0.5–1 μg of the *C. elegans* cDNA library as previously described [28] and transformants were selected on TRP minimal media Petri